

function as antigen presenting cells, and could explain the observed relation between viral and allogeneic immunity.

## P20

### HERPES VIRUSES ORAL SHEDDING IN CHRONIC RENAL PATIENTS

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**Introduction:** Chronic renal failure (CRF) patients present inability of the kidney to maintain normal product of protein metabolism, blood pressure and hematocrit, and also the control of sodium, water, potassium and acid-base balance. Cellular and humoral immune response and can be depressed so the existence of disorders in oral health may represent focus of infection. According the literature patients under hemodialysis have a 40-50-fold increased risk of mortality from sepsis compared with the general population. The monitoring of opportunistic viruses is an extreme importance, since they are related to comorbidities in these individuals. The objective of this study was to analyze the presence of human herpesviruses (HSV1, HSV2, VZV, EBV, CMV, HHV6, HHV7 and HHV8) in saliva of CRF patients.

**Methods:** 105 individuals divided into 3 groups according to the stage of renal disease. Group 1 (n=13) control group; Group 2 (n=53) patients with chronic renal disease and group 3 (n=33) end-stage renal disease (ESRD) patients in hemodialysis. For saliva collection was used the technique of salivary flow non-stimulated. Samples were evaluated by multiplex PCR for herpesviruses.

**Results:** The herpesviruses CMV, HSV-2, and HHV-8 were not found in any of the saliva samples. The distribution of herpesviruses in the study patients is shown in Table 1. The comparison of the prevalence of HSV-1 between group 3 and 2 showed significant difference ( $p = 0.0307$ ). EBV, VZV, HHV-6 and HHV-7 did not show any difference.

Table 1. Distribution of herpesviruses in the 3 groups (number of patients and percentage)

GROUP	N	NEGATIVE	HSV-1	EBV	VZV	HHV-6	HHV-7
1	13	3 (23.07)	1 (7.69)	7 (53.84)	0	1 (7.69)	7 (53.84)
2	53	18 (33.96)	4 (7.54)	25 (47.16)	3 (5.66)	4 (7.54)	26 (49.05)
3	33	9 (27.27)	8 (24.24)	18 (54.54)	4 (12.12)	1 (3.03)	17 (51.51)

The most prevalent co-infection was EBV/HHV-7 detected in 40% of all patients. The distribution of the co-infection of the 3 groups are shown in Table 2. The statistical analysis did not show difference between the groups, but we detected the association of the HSV-1/EBV/HHV-7 just in the group 3.

Table 2 - Herpesviruses co-infection in saliva samples (number of patients and percentage).

GROUP	N	HSV-1/EBV	EBV/HHV-7	EBV/HHV-6/HHV-7	EBV/VZV/HHV-7	HSV-1/EBV/HHV-7
1	10	1 (10.00)	3 (30.00)	1 (10.00)	0	0
2	35	1 (2.85)	15 (42.85)	15 (42.85)	2 (5.71)	0
3	24	2 (8.33)	5 (20.83)	5 (20.83)	2 (8.33)	5 (20.83)

**Conclusions:** With the results obtained we can conclude that serological screening for herpesvirus in chronic renal failure

patients and hemodialysis, which are transplant candidates is essential to determine its been immune. The prevalence of co-infection in hemodialysis patients as HSV -1 / EBV / HHV -7 can cause complications in these patients.

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## P21

### BETA-HYDROXYBUTYRATE INHIBITS NLRP3-MEDIATED INFLAMMATION AND DELAYS PROGRESSIVE RENAL FAILURE DURING PRIMARY HYPEROXALURIA RELATED KIDNEY STONE DISEASE

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**Introduction:** Primary hyperoxaluria is a condition characterized by overproduction of oxalate. When available in excess, oxalate combines with calcium to form crystals of calcium oxalate leading to kidney stone disease. NLRP3 inflammasome-mediated renal inflammation was identified as a prime pathomechanism of acute oxalate nephropathy (Mulay et. al., JCI 2013) as well as kidney stone disease (Knauf et. al., KI 2013). In addition, a recent report demonstrated that  $\beta$ -hydroxybutyrate (BHB) inhibited NLRP3-mediated inflammation (Youn YH et. al., Nat Med 2015). Therefore, we speculated that BHB would ameliorate calcium oxalate (CaOx) crystals-induced NLRP3-mediated inflammation in acute oxalate nephropathy as well as primary hyperoxaluria related kidney stone disease.

**Methods:** C57BL/6 mice at the age of 6-to 8-week old were purchased from Charles River, which received a single intraperitoneal injection of 100 mg/kg sodium oxalate for the acute calcium oxalate nephropathy model and kidneys were harvested after 24 hours. For the chronic model, mice were fed with a low calcium plus Na-Oxalate diet. In both models, mice were fed with a ketogenic diet (KD) containing 1,3 butanediol ketone diesters for 5-7 days before induce kidney damage. Urine and blood samples were collected at different time intervals (before and at 3, 7 and 14 days after damage) and kidneys were used for RNA isolation and histological analysis (IHC). The kidney function was quantified by the glomerular filtration rate (GFR), plasma creatinine and BUN levels at indicated times. Markers of kidney damage (Kim-1), inflammation (IL-6, MCP-1, RANTES, TNF-) and fibrotic markers (SMA, Col1A1, FSP-1, Fib-1) were analyzed by RT-PCR and IHC. The BHB levels were assayed in serum samples with a colorimetric assay kit (Cayman Chemicals, MI, USA).

**Results:** We observed that BHB inhibited CaOx crystals-induced NLRP3-mediated IL-1 $\beta$  release from bone marrow derived dendritic cells in a dose dependent manner in vitro. Moreover, feeding of ketogenic diet (KD) to C57BL6 mice elevated their plasma BHB levels and protected them from acute oxalate nephropathy as seen by decreased plasma BUN and creatinine levels despite similar renal crystal deposition. KD also reduced tubular injury (PAS, Kim-1), renal neutrophil infiltration and renal inflammation (IL-6, TNF, RANTES and CXCL2) during acute oxalate nephropathy. Similarly, KD did not change the renal CaOx crystal deposition and plug formation during kidney stone disease, however still protected mice from progressive renal failure as seen by improvement in glomerular filtration rate (GFR) and decreased plasma BUN and creatinine levels. Elevated plasma BHB levels after KD administration

correlated with the reduction of tubular injury (PAS, Kim-1), renal inflammation (Macrophages, T cells, IL-6, TNF-, RANTES and CCL2) as well as renal fibrosis (SMA, Colla1, Masson Trichrome).

**Conclusions:** Therefore, we conclude that BHB ameliorates acute oxalate nephropathy, as well as delays progressive renal failure by inhibiting NLRP3-mediated inflammation. Our findings suggest that ketogenic diets or -hydroxybutyrate formulations can be used therapeutically for the treatment of primary hyperoxaluria related kidney stone disease.

## P22

### A NOVEL METHOD FOR HIGH-THROUGHPUT DETECTION AND QUANTIFICATION OF NEUTROPHIL EXTRACELLULAR TRAPS REVEALS ROS-INDEPENDENT NET RELEASE WITH IMMUNE COMPLEXES

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**Introduction:** A newly-described 1st-line immune defence mechanisms of neutrophils is the extrusion of DNA, called neutrophil extracellular traps (NETs). Known non-infectious triggers of NETosis are phorbol 12-myristate 13-acetate (PMA), IL-8 and immune complexes (ICx). In vitro, PMA and IL-8 are strong inducers of NET release and generally involves all cultured neutrophils. However, ICx induce much lower numbers of neutrophils to release NETs. As such, the in vitro quantification of NETs is challenging with current methodologies (i.e. Picogreen or immunofluorescence). In order to investigate the role of NET release in ICx-mediated autoimmune diseases, we developed a novel method for automated and highly sensitive measurement of NETs, applicable in a high-throughput system.

**Methods:** Healthy neutrophils are isolated from fresh blood and labelled with the membrane staining PKH26, which serves as a cell marker. Neutrophils are stimulated for 4 hours with various concentrations of heat-aggregated human IgG ICx. After 3<sup>3</sup>/<sub>4</sub> hours, Sytox green is added to stain extracellular DNA. Sytox green is incubated for 15 minutes, after which the cells are fixed with 4% PFA. Within 24 hours, the 96-well plate is automatically imaged with the BD Pathway 855. The ratio of the sum of Sytox green area and average PKH area is calculated, representing the amount of NETs in each sample. A higher ratio indicates a higher amount of extracellular DNA present.

**Results:** Immunofluorescence analysis of ICx-stimulated neutrophils revealed colocalization of extracellular DNA staining with citrullinated histone3 and neutrophil elastase, confirming the release of NETs. The amount of NET release was quantified with ImageJ software, by determining the ratio between positive Sytox green area and the positive PKH26 area, of which we found that it accurately represents the amount of cells imaged. Sensitivity is highly increased since 11% of the total area per well is imaged and a z-stacking system is used to image NETs superimposed on neutrophils. We further characterized NET release upon stimulation with these ICx. When pre-treated with diphenyleneiodonium (DPI), used to block production of reactive oxygen species (ROS), NET release upon ICx stimulation was not affected.

**Conclusions:** We developed a novel assay that is highly sensitive to detect and quantify in vitro NET release. NET release triggered by human ICx was detected reliably and appeared to be a ROS-independent process. This assay will allow high-throughput and highly sensitive analysis of NET release in ICx-mediated autoimmune diseases (e.g. systemic lupus erythematosus, rheumatoid arthritis or cryoglobulinemic vasculitis).

## P23

### IL-33 ELICITS INNATE LYMPHOID CELLS AND ALTERNATIVELY ACTIVATED MACROPHAGES THAT REDUCE RENAL ISCHEMIC-REPERFUSION INJURY

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**Introduction:** IL-33 is an important immune regulator which can promote Th2 immune response-dependent immunity, inflammation, and tissue repair in several important immune-mediated disorders. In the current study, we sought to determine whether IL-33 is an important regulator in renal ischaemic/reperfusion injury (IRI).

**Methods:** Bilateral renal ischemia was imposed in C57BL/6 mice under isoflurane anesthesia. IL-33 was given by 5 consecutive daily injections starting at day 5 before IRI operation. Adoptive transfer of type 2 innate lymphoid cells into mice with IRI was used to assess their in vivo functions.

**Results:** Treating IRI mice with IL-33 significantly improved renal function and reduced renal injury. The possible mechanisms underlying the protective effect of IL-33 were examined. Firstly, IL-33 increased the levels of IL-4, IL-5 and IL-13 in serum and kidney and promoted induction of alternatively activated (M2) macrophages in kidney. Moreover, the number of NK cells and neutrophils was significantly reduced in IRI mice treated with IL-33. A novel finding of this study was that IL-33 increased the frequency of type 2 innate lymphoid cells (ILC2) and regulatory T cells (Tregs) in kidney. However, adoptive transfer of ILC2 not only reduced renal functional and histological injury in IRI mice but also induced M2 macrophages in kidney.

**Conclusions:** In conclusion, our data identify a mechanism whereby IL-33 eliciting ILC2 and Tregs regulate macrophage phenotype in kidney and prevent renal IRI.

## P24

### ROR<sup>γ</sup>t<sup>+</sup> FOXP3<sup>+</sup> BITREGS PROMOTE LUPUS NEPHRITIS VIA IL-17 SECRETION AND SUPPRESSION OF TH2 RESPONSES

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**Introduction:** We have recently characterized Foxp3<sup>+</sup> regulatory T cells, co-expressing the Th17 characteristic transcription factor ROR t, as an independent and bi-functional T cell lineage (biTregs). biTregs secrete both, anti-inflammatory (IL-10, IL-35), but also pro-inflammatory (IL-17) cytokines. Studies in a model of acute crescentic glomerulonephritis have suggested that pro-inflammatory biTreg functions are largely mediated by ROR t. This is of high clinical relevance, since multiple ROR t blocking agents are